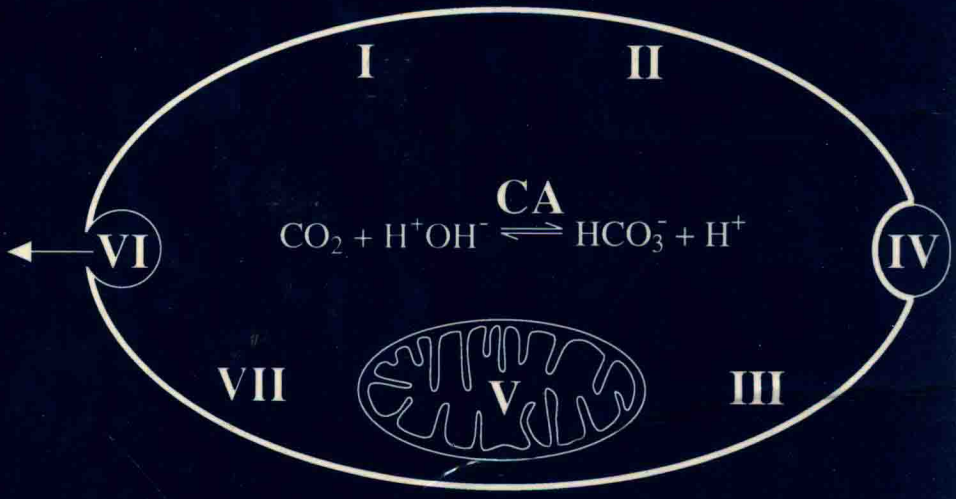
The Carbonic Anhydrases

Cellular Physiology and Molecular Genetics



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Dedication

The contributions of Robert Elder Forster II to the understanding of the physiological functions of the carbonic anhydrases are many. He has always been a gifted tinkerer and delights in theoretical derivations of complicated mathematical models. During his tenure as Chairman of the Department of Physiology at the University of Pennsylvania (1959 – 1990), he provided a stimulating laboratory environment for junior scientists as well as for collaborations with colleagues. His interest in carbon dioxide homeostasis and pH continues unabated.

He was the first of three sons of a Philadelphia lawyer, born in time to remember Prohibition and his father's futile attempts at circumventing those dry days. The Spanish Civil War fired his ideological zeal, and perhaps this dedication could have ended there but for the refusal of his parents to let their teenager sign up. He was sent to Yale in 1937 and did well enough to be accepted into medical school at the University of Pennsylvania in 1940. The war effort left the nation's supply of physicians depleted: the university responded by offering a three-year M.D. program. After graduating in 1943, he interned in Boston at the Peter Bent Brigham Hospital, spent two years as a captain in the Quartermaster Corps in the Climatic Research Laboratory, and returned to Boston for a residency. He then settled down to become a physiologist, starting at Harvard under Eugene Landis and then returning to the University of Pennsylvania under the guidance of Julius J. Comroe, Jr.

Since his first publication in 1943, Dr. Forster has produced many reports concerning blood flow and gas exchange. It was his interest in gas exchange that brought him renown in the field of alveolar ventilation and also took him to his long-abiding interest in carbon dioxide and the carbonic anhydrases. He was the senior editor on the first book of collected papers about carbon dioxide and the carbonic anhydrases, which came out of a conference he organized at Haverford College in 1968. A collaboration and

friendship with Professor F. J. W. Roughton, who is listed as a coeditor on the first carbonic anhydrase book, lasted long before and until Professor Roughton's death in 1971. Dr. Forster's many papers reflect his interest in developing new techniques. His review article in this book discusses every technique he could find which has been used to measure carbonic anhydrase activity; many of these techniques he developed himself.

This book includes papers from former and present students and colleagues: G. Gros, R. P. Henry, S. F. Silverton, B. T. Storey, S. Lahiri, and S. J. Dodgson. Others still in the field include E. D. Crandall and A. Bidani.

This dedication would not be complete without the acknowledgment of the love and comfort provided by Elizabeth Hilbert Forster (née Day) since their wedding in 1947. His beloved Betsy has also considerably aided quite a lot of us, both in her capacity as a psychiatric social worker and as a friend. What a great team.

Susanna J. Dodgson

No single individual, with the possible exception of F. J. W. Roughton, is more closely linked with carbonic anhydrase than Thomas H. Maren. Indeed, the affinity is so strong that even he himself has found the bond difficult to break. Although in dedicating this book to him, it is his many contributions toward our understanding of the enzyme that we celebrate, he has also figured prominently in other areas of pharmacology and medical education.

Surveying his three and a half decades of research in carbonic anhydrase, one readily appreciates both the depth and breadth of his investigative mind. His contributions include many fundamental studies into the physical chemistry, biochemistry, physiology, and pharmacology of the enzyme and its inhibitors. There is hardly a facet of carbonic anhydrase that has escaped his curiosity or not yielded to his theoretical and experimental skills. He came to focus on the enzyme relatively later on in his scientific career, after formal training in chemistry and English literature at Princeton and then in pharmacology, physiology, and medicine at Johns Hopkins. In the war years, between his undergraduate and medical education, there was further practical research experience gained first as a chemist in a small cosmetics firm and then in parasitology at the School of Public Hygiene at Hopkins. Such a broad grounding in the sciences and humanities set the stage and surely helps to explain his unmatched productivity and wide-ranging investigations on the enzyme. Since his earliest work in the 1950s that led to the clinical introduction of acetazolamide, carbonic anhydrase has been for him a scientific passion and portal into the joys of experimental chemistry,

physiology, pharmacology, and medicine. He has elucidated the quantitative relationship between the chemical reaction rates of the enzyme and the physiological rates of CO_2 and acid–base transport in a host of tissues. This has led to a much better understanding of the physiological roles of carbonic anhydrase in gas exchange and fluid secretion, to a fuller understanding of the clinical consequences of enzyme inhibition, and in some cases to the possibility of important carbonic anhydrase–independent modes of acid–base movement. His pioneering work on acetazolamide, which found its greatest application in the treatment of glaucoma, has kept him in the forefront of basic research in ophthalmology. Thus, it is somewhat ironic but entirely fitting that his most recent work has closed a full circle. His laboratory has successfully synthesized new and effective topical carbonic anhydrase inhibitors with intraocular pressure–lowering effects equal to those of acetazolamide, whose systemic side effects preclude its use in many patients. By his own hand, the drug which he so successfully launched at the start of his career will likely be retired into relative clinical obscurity.

To conclude this brief tribute to Tom, I wish to express my deepest gratitude and affection to him. As mentor, collaborator, and friend, he has by his wisdom, integrity, humor, curiosity, and compassion provided an inspiring example of the best human synthesis of science and the arts. I believe that all who have had the honor and pleasure of working with him would share these sentiments.

Erik Swenson

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Preface

As we approach the twenty-first century the problems of industrialization are evident: we find there is a greenhouse effect, the ozone layer is being depleted, the rain is acidified, and there is a terrible problem of increasing CO_2 concentrations in the atmosphere. The carbonic anhydrases are a unique family of enzymes that solve these problems in the human body: they are responsible for converting CO_2 (a gas) to HCO_3^-, which is the biggest intracellular buffer, with a concomitant decrease in a hydroxyl ion. Globally, the functions of the carbonic anhydrases in photosynthesis in rain forests and in the algae and plankton that cover our oceans indicate that they are also of utmost importance in the maintenance of the acid–base balance on our planet. Although the whole field of CO_2 metabolism is enormous and still rapidly expanding, because of the research interests of the editors this book is mainly concerned with mammalian carbonic anhydrases. However, if the interested reader intends to purify carbonic anhydrases from nonmammalian sources, Dr. Chegwidden has provided the necessary information in Chapter 7.

The carbonic anhydrases were first discovered in 1933; until 1976 there were thought to be only two isozymes. Since then CA III, IV, V, VI, and VII have been discovered and well characterized. There is, of course, no reason to believe that we have found them all. Certainly, a carbonic anhydrase sitting on a plasma membrane (CA IV) would be expected to function under entirely different conditions than one floating freely in an erythrocyte (CA II) or one in a mitochondrial matrix (CA V); however, this certainty has only come with hindsight and with the huge advance in knowledge of cellular physiology. The idea of copious quantities of a carbonic anhydrase being produced just to be secreted in the saliva (CA VI) sounds absurd; Dr. Fernley has isolated the cDNA clone (Chapter 34). The carbonic anhydrases, particularly CA II, are well known as being those enzymes with the highest turnover numbers of any found in nature. Perhaps there are a plethora of low-turnover-number carbonic anhydrase isozymes which have so far remained undetected, of which CA III is the first example, or a whole group of acetazolamide-insensitive isozymes, of which, again, CA III is so far the only mammalian example.

It was first discovered in 1940 that some sulfonamide drugs are specific carbonic anhydrase inhibitors. Acetazolamide has been used successfully to treat

glaucoma since the early 1950s: the importance of carbonic anhydrase inhibitors in ophthalmology is described by Drs. Feitl and Krupin (Chapter 13) and the discovery and pharmacology of topical carbonic anhydrase inhibitors is described by Dr. Lippa (Chapter 14). Studies with other organs described in this book led to the conclusion that mostly activation, rather than inhibition, of carbonic anhydrases is desirable. This conclusion is certainly reached after consideration of the enormous medical problems of patients lacking just one isozyme, CA II, as described by the discoverer of the CA II-deficiency syndrome, Dr. Sly (Chapter 15).

When I explain to colleagues that there are several carbonic anhydrase isozymes eyes light up. They glitter when I explain that the different isozymes are mainly products of different genes. Nature could not spend so much energy on a family of unnecessary enzymes. A major aim of this book is to bring together information about the individual isozymes, from gene sequencing, crystallographic structures and kinetics of the isolated organs, to their functioning in intact organs. We know a great deal more about this family of isozymes than in 1984 when Dr. Tashian and Dr. Hewett-Emmett edited the previous compendium of reviews (*Annals New York Academy of Sciences*, volume 429, 1984). Another major aim of this book is to present details of common techniques used by workers in the field so that any interested scientist can easily set up assays for carbonic anhydrase activity, and immunohistochemical or histochemical visualization. The special problems of carbonic anhydrases in cultured cells are addressed by Dr. Venta (Chapter 5).

To those understanding only the meaning of "the" and "and" in the title of this book, I explain this is an anthology of the wonderful things that can happen throughout the body when a gas becomes a salt, with recipes. I explain that this process is so fundamental that, without it, life is probably impossible. To these intrepid and persevering readers I refer especially to Chapters 1 and 2, which give a general outline of the field from physiological and genetic perspectives.

This book has the distinction of being the first exclusively dedicated to the carbonic anhydrases which is not the proceedings of a conference. This has not been an easy task and there are many people to thank. My older sons Angus Z. Dodgson Pekala and Miles C. Dodgson Pekala have helped immensely by amusing my youngest son, Allister M. Dodgson Blossfeld while I was glued to the computer. Miss Karen Lapps and Dr. Fernley helped with the design of the front cover, which ultimately was executed by Dr. Tashian and his staff. The staff at Plenum has been helpful and pleasant; without Mrs. Mary Phillips Born there would not be a book. Each chapter was reviewed by up to four colleagues; Dr. Storey's help was particularly commendable. I would also like to thank the co-editors, who are all my good friends and colleagues, for their input. Since it is cheaper to call Ann Arbor than Germany or England, Dr. Tashian has been a most frequent listener and arbiter of disputes.

Susanna J. Dodgson

Philadelphia

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PART I

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